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(57) Abstract			
<p>Human GPR14 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing Human GPR14 polypeptides and polynucleotides in the design of protocols for the treatment of ischemic coronary artery disease (angina and myocardial infarction); atherosclerosis; metabolic diseases (e.g. diabetes); CHF/myocardial dysfunction; arrhythmias; restenosis; hypertension; hypotension; pulmonary disease (hypertension, COPD, asthma); fibrotic vasculopathies (diabetes, SLE, AS, Reynaud's); cerebrovascular events (e.g. hemorrhagic and ischemic stroke); neurogenic inflammation/migraine; hematopoietic disorders; ARDS; cancer; autoimmune diseases (e.g. HIV-1 and -2 infection and AIDS); gastrointestinal and genitourinary disturbances (e.g. ulcers); endocrine disorders; fibroproliferative disorders (e.g. psoriasis); inflammatory disease (e.g. RA, Crohn's, IBS); benign prostatic hypertrophy; renal failure and glomerulopathies; disease states, both cardiovascular and non-cardiovascular, which are characterized by excessive vasoconstriction, myocardial dysfunction and/or aberrant fibroproliferative/inflammatory responses; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation, Parkinson's disease, and dyskineticias, infections such as bacterial, fungal, protozoan and viral infections; pain; eating disorders, such as obesity, anorexia, and bulimia; asthma; urinary retention; osteoporosis; allergies; Huntington's disease or Gilles de la Tourette's syndrome, among others and diagnostic assays for such conditions.</p>			

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HUMAN RECEPTOR GPR14, AND A METHOD OF FINDING AGONIST AND ANTAGONIST TO HUMAN AND RAT GPR14

This application claims the benefit of priority from U.S. Provisional Application Serial No. 60/074, 075 filed February 9, 1998 and U.S. Continuation-In-Part Application 5 Serial No. *to be provided* filed January 15, 1999, which, in turn, claims priority of Continuation-In-Part Application Serial No. 09/058,725 filed April 10, 1998. All three applications are incorporated herein by reference in their entireties.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by 10 them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to G-Protein coupled receptor, hereinafter referred to as Human GPR14. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

15 It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers, e.g., cAMP (Lefkowitz, Nature, 1991, 351:353-354). Herein these proteins are referred to as proteins participating in pathways with G-proteins or PPG proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and 20 dopamine (Kobilka, B.K., et al., Proc. Natl Acad. Sci., USA, 1987, 84:46-50; Kobilka, B.K., et al., Science, 1987, 238:650-656; Bunzow, J.R., et al., Nature, 1988, 336:783-787), G-proteins themselves, effector proteins, e.g., phospholipase C, adenyl cyclase, and phosphodiesterase, and actuator proteins, e.g., protein kinase A and protein kinase C (Simon, M.I., et al., Science, 1991, 252:802-8).

25 For example, in one form of signal transduction, the effect of hormone binding is activation of the enzyme, adenylate cyclase, inside the cell. Enzyme activation by hormones is dependent on the presence of the nucleotide, GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylate cyclase. G-protein was shown to exchange GTP for bound GDP when activated by a hormone receptor. The GTP-carrying

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form then binds to activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

5 The membrane protein gene superfamily of G-protein coupled receptors has been characterized as having seven putative transmembrane domains. The domains are believed to represent transmembrane α -helices connected by extracellular or cytoplasmic loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

10 G-protein coupled receptors have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. The G-protein family of coupled receptors includes dopamine receptors which bind to neuroleptic drugs used for treating psychotic and neurological disorders. Other examples of members of this family include, but are not limited to, calcitonin, adrenergic, endothelin, cAMP, adenosine, muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorant, and cytomegalovirus receptors.

15 Most G-protein coupled receptors (or otherwise known as 7TM receptors) have single conserved cysteine residues in each of the first two extracellular loops which form disulfide bonds that are believed to stabilize functional protein structure. The 7 transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

20 Phosphorylation and lipidation (palmitylation or farnesylation) of cysteine residues can influence signal transduction of some G-protein coupled receptors. Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the β -adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

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For some receptors, the ligand binding sites of G-protein coupled receptors are believed to comprise hydrophilic sockets formed by several G-protein coupled receptor transmembrane domains, said sockets being surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson et al., Endoc. Rev., 1989, 10:317-331). Different G-protein α -subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors has been identified as an important mechanism for the regulation of G-protein coupling of some G-protein coupled receptors.

G-protein coupled receptors are found in numerous sites within a mammalian host.

Over the past 15 years, nearly 350 therapeutic agents targeting 7 transmembrane (7 TM) receptors have been successfully introduced onto the market.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, ischemic coronary artery disease (angina and myocardial infarction); atherosclerosis; metabolic diseases (e.g. diabetes); CHF/myocardial dysfunction; arrhythmias; restenosis; hypertension; hypotension; pulmonary disease (hypertension, COPD, asthma); fibrotic vasculopathies (diabetes, SLE, AS, Reynaud's); cerebrovascular events (e.g. hemorrhagic and ischemic stroke); neurogenic inflammation/migraine; hematopoietic disorders; ARDS; cancer; autoimmune diseases (e.g. HIV-1 and -2 infection and AIDS); gastrointestinal and genitourinary disturbances (e.g. ulcers); endocrine disorders; fibroproliferative disorders (e.g. psoriasis); inflammatory disease (e.g. RA, Crohn's, IBS); benign prostatic hypertrophy; renal failure and glomerulopathies; disease states, both cardiovascular and non-cardiovascular, which are characterized by excessive vasoconstriction, myocardial dysfunction and/or aberrant fibroproliferative/inflammatory

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responses; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation, Parkinson's disease, and dyskinesias, infections such as bacterial, fungal, protozoan and viral infections; pain; eating disorders, such as obesity, anorexia, and bulimia; asthma; urinary retention; osteoporosis; 5 allergies; Huntington's disease or Gilles dela Tourett's syndrome.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to Human GPR14 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such Human GPR14 polypeptides and polynucleotides. Such uses include 10 the treatment of ischemic coronary artery disease (angina and myocardial infarction); atherosclerosis; metabolic diseases (e.g. diabetes); CHF/myocardial dysfunction; arrhythmias; restenosis; hypertension; hypotension; pulmonary disease (hypertension, COPD, asthma); fibrotic vasculopathies (diabetes, SLE, AS, Reynaud's); cerebrovascular events (e.g. hemorrhagic and ischemic stroke); neurogenic inflammation/migraine; 15 hematopoietic disorders; ARDS; cancer; autoimmune diseases (e.g. HIV-1 and -2 infection and AIDS); gastrointestinal and genitourinary disturbances (e.g. ulcers); endocrine disorders; fibroproliferative disorders (e.g. psoriasis); inflammatory disease (e.g. RA, Crohn's, IBS); benign prostatic hypertrophy; renal failure and glomerulopathies; disease states, both cardiovascular and non-cardiovascular, which are characterized by excessive 20 vasoconstriction, myocardial dysfunction and/or aberrant fibroproliferative/inflammatory responses; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation, Parkinson's disease, and dyskinesias, infections such as bacterial, fungal, protozoan and viral infections; pain; eating disorders, such as obesity, anorexia, and bulimia; asthma; urinary retention; osteoporosis; 25 allergies; Huntington's disease or Gilles dela Tourett's syndrome, among others.

In accordance with another aspect of the present invention there are provided methods of screening for compounds which bind to and activate (agonist) or inhibit activation (antagonist) of rat or Human GPR 14 polypeptides (receptors), and for their ligands.

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In particular, the preferred method for identifying agonist or antagonist of a Human or rat GPR 14 polypeptide comprises:

contacting a cell expressing on the surface thereof the polypeptide, said polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and

determining whether the compound binds to and activates or inhibits the polypeptide by measuring the level of a signal generated from the interaction of the compound with the polypeptide.

10 In a further preferred embodiment, the method further comprises conducting the identification of agonist or antagonist in the presence of labeled or unlabeled, fish or Human urotensin II.

15 In another embodiment of the method for identifying agonist or antagonist of a human or rat GPR 14 polypeptide comprises:

determining the inhibition of binding of a ligand to cells which have the polypeptide on the surface thereof, or to cell membranes containing the polypeptide, in the presence of a candidate compound under conditions to permit binding to the polypeptide, and determining the amount of ligand bound to the polypeptide, such that a compound capable of causing reduction of binding of a ligand is an agonist or antagonist. Preferably, the ligand is human or rat urotensin II. Yet more preferably human or rat urotensin II is labeled.

20 Further the present invention relates to treating conditions associated with Human GPR14 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate Human GPR14 activity or levels.

25 BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1, 1A and 1B show the nucleotide and deduced amino acid sequence of Human GPR14. SEQ ID NOS: 1 and 2.

Figures 2A and 2B show the amino acid sequence and polynucleotide sequence of rat GPR14 receptor. SEQ ID NO: 4 and 6.

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Figure 3 shows concentration response curves for fish urotensin II against HEK 293 rat and Human GPR14 transients + G alpha-16 using FLIPR.

Figure 4 is the concentration-response-curves for human urotensin II for 3 clones of the Human GPR 14 stably transfected cells.

5 DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"Human GPR14" refers generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

"Fish urotensin II" refers to polypeptide with amino acid sequence of Ala-Gly-Thr-Ala-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val (SEQ ID NO:3), which is described by Pearson et al., in Proc Natl Acad Sci U S A 1980 Aug;77(8):5021-5024.

"Rat GPR14 receptor" or "rat GPR 14 receptor polypeptide" is a protein with GenBank Accession number U32673, and described by Marchese et al., in Genomics 1995 Sep 20;29(2):335-344. Rat GPR14 receptor has amino acid sequence shown in Figure 2 (SEQ ID NO: 4).

"Human urotensin II" is a protein with the amino acid sequence ETPDCFWKYCV (SEQ ID NO: 5). Our studies have now found that Human urotensin II of SEQ ID NO: 5 to be the most potent mammalian vasoconstrictor identified to date and, in the intact primate, induces systemic vasoconstriction, myocardial contractile dysfunction and, ultimately, lethal arrhythmias (additional metabolic/endocrine dysfunction may also result from excessive activity of this system). "Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said Human GPR14 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said Human GPR14.

"Human GPR14 polypeptides" refers to polypeptides with amino acid sequences sufficiently similar to Human GPR14 sequences, preferably exhibiting at least one biological activity of the receptor.

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"Human GPR14 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Human GPR14 polynucleotides" refers to polynucleotides containing a nucleotide sequence which encodes a Human GPR14 polypeptide or fragment thereof, or a 5 nucleotide sequence which has at least 75.9 % identity to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or the corresponding fragment thereof, or a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker.

10 "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

15 "Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

20 "Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double- 25 stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases 30 such as inosine. A variety of modifications has been made to DNA and RNA; thus,

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"polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

5 "Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids.

10 "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications.

15 Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods.

20 Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York,

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1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and 5 Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from 10 another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence 15 from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic 20 code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" as known in the art, is a relationship between two or more polypeptide 25 sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational* 30 *Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press,

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New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math.*, 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990)). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990)). The well known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following:

1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA*. 89:10915-10919 (1992)

20 Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

25 Preferred parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

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Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

Preferred polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO: 1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or: $n_n \times_n - (x_n \cdot y)$, wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and \cdot is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one nucleic acid deletion, substitution, , including transition and transversion, or insertion, and wherein said

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alterations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleic acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleic acid alterations for a given percent identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or: $n_n = x_n - (x_n \cdot y)$, wherein 5 n_n is the number of amino acid alterations, x_n is the total number of amino acids in SEQ ID NO:2, y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., , is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y 10 is rounded down to the nearest integer prior to subtracting it from x_n .

Preferred polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said polypeptide sequence 15 may be identical to the reference sequence of SEQ ID NO: 2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal 20 positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 25 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or: $n_a = x_a - (x_a \cdot y)$, wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 30 for 100%, and is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

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By way of example, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is it may be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such
5 alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one
10 or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:
 $n_a = x_a - (x_a \cdot y)$,

15 wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

Polypeptides of the Invention

20 The Human GPR14 polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) as well as Human GPR14 polypeptides and which have at least 84.25 % identity to the polypeptide of SEQ ID NO:2 or the relevant portion and more preferably at least 85 % identity, and still more preferably at least 90% identity, and even still more preferably at least 95 % identity to
25 SEQ ID NO: 2.

The Human GPR14 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or
30 an additional sequence for stability during recombinant production.

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Biologically active fragments of the Human GPR14 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned Human GPR14 polypeptides. As with Human GPR14 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of Human GPR14 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 10 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of Human GPR14 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Thus, the polypeptides of the invention include polypeptides having an amino acid sequence at least 84.25 % identical to that of SEQ ID NO:2 or fragments thereof with at least 84.25 % identity to the corresponding fragment of SEQ ID NO:2. Preferably, all of these polypeptides retain the biological activity of the receptor, including antigenic activity. Included in this group are variants of the defined sequence and fragments. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic

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residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

5 The Human GPR14 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

10 Another aspect of the invention relates to isolated polynucleotides which encode the Human GPR14 polypeptides and polynucleotides closely related thereto.

Human GPR14 of the invention is structurally related to other proteins of the G-Protein coupled receptor, as shown by the results of sequencing the cDNA encoding Human GPR14. The cDNA sequence contains an open reading frame encoding a protein of 390 15 amino acids. Human GPR14 of Figure 1 (SEQ ID NO:2) has about 84.25 % identity (using Fasta) in 387 amino acid residues with Rattus norvegicus GPR14 orphan receptor (A. Marches, et al., Genomics 29(2):335-344, 1995). Furthermore, human GPR14 (SEQ. ID NO:2) is 31.7% identical to human Somatostatin-3 receptor over 331 amino acid residues (Y. Yamada et al., Mol. Endocrinol. 6(12):2136-2142 , 1992). Human GPR14 gene of 20 Figure 1 (SEQ ID NO:1) has about 75.9 % identity (using blast) in 1539 bp nucleotide residues with Rattus norvegicus GPR14 orphan receptor (A. Marches, et al., Genomics 29(2): 335-344, 1995). Furthermore, human GGPR14(SEQ. ID NO:1) is 79 % identical over 783bp to Rattus norvegicus G- protein coupled receptor SENR (M. Tal, et al., Biochem. Biophys. Res. Commun. 209(2):752-759 1995.)

25 One polynucleotide of the present invention encoding Human GPR14 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human placenta using the expressed sequence tag (EST) analysis (Adams, M.D., et al. *Science* (1991) 252:1651-1656; Adams, M.D. et al., *Nature*, (1992) 355:632-634; Adams, M.D., et al., *Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention

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can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

Thus, the nucleotide sequence encoding Human GPR14 polypeptides may be identical over its entire length to the coding sequence in Figure 1 (SEQ ID NO:1), or may 5 be a degenerate form of this nucleotide sequence encoding the polypeptide of SEQ ID NO:2, or may be highly identical to a nucleotide sequence that encodes the polypeptide of SEQ ID NO:2. Preferably, the polynucleotides of the invention contain a nucleotide sequence that is highly identical, at least 75.9% identical, with a nucleotide sequence encoding a Human GPR14 polypeptide, or at least 75.9% identical with the encoding 10 nucleotide sequence set forth in Figure 1 (SEQ ID NO:1), or at least 75.9% identical to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of Human GPR14 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature 15 polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector 20 (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Among particularly preferred embodiments of the invention are polynucleotides 25 encoding Human GPR14 polypeptides having the amino acid sequence of set out in Figure 1 (SEQ ID NO:2) and variants thereof.

Further preferred embodiments are polynucleotides encoding Human GPR14 variants that have the amino acid sequence of the Human GPR14 of Figure 1 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, 30 in any combination.

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Further preferred embodiments of the invention are polynucleotides that are at least 75.9 % identical over their entire length to a polynucleotide encoding the Human GPR14 polypeptide having the amino acid sequence set out in Figure 1 (SEQ ID NO:2), and polynucleotides which are complementary to such polynucleotides. In this regard, 5 polynucleotides at least 80 % identical over their entire length to the same are particularly preferred, and those with at least 90% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred.

The present invention further relates to polynucleotides that hybridize to the herein 10 above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95 % and preferably at least 97% identity between the sequences.

15 Polynucleotides of the invention, which are sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding Human GPR14 and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the Human GPR14 gene. Such hybridization techniques are known to those of skill in the 20 art. Typically these nucleotide sequences are 70% identical, preferably 80% identical, more preferably 90% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

25 The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

30 The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered

with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention.

Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

(1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL (supra)*.

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For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

5 If the Human GPR14 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If Human GPR14 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before
10 the polypeptide is recovered.

Human GPR14 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite
15 chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

20 This invention also relates to the use of Human GPR14 polynucleotides for use as diagnostic reagents. Detection of a mutated form of Human GPR14 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of Human GPR14. Individuals carrying mutations in the Human GPR14 gene
25 may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar
30 fashion. Deletions and insertions can be detected by a change in size of the amplified

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product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled Human GPR14 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401.

The diagnostic assays offer a process for diagnosing or determining a susceptibility to ischemic coronary artery disease (angina and myocardial infarction); atherosclerosis; metabolic diseases (e.g. diabetes); CHF/myocardial dysfunction; arrhythmias; restenosis; hypertension; hypotension; pulmonary disease (hypertension, COPD, asthma); fibrotic vasculopathies (diabetes, SLE, AS, Reynaud's); cerebrovascular events (e.g. hemorrhagic and ischemic stroke); neurogenic inflammation/migraine; hematopoietic disorders; ARDS; cancer; autoimmune diseases (e.g. HIV-1 and -2 infection and AIDS); gastrointestinal and genitourinary disturbances (e.g. ulcers); endocrine disorders; fibroproliferative disorders (e.g. psoriasis); inflammatory disease (e.g. RA, Crohn's, IBS); benign prostatic hypertrophy; renal failure and glomerulopathies; disease states, both cardiovascular and non-cardiovascular, which are characterized by excessive vasoconstriction, myocardial dysfunction and/or aberrant fibroproliferative/inflammatory responses; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation, Parkinson's disease, and dyskinesias, infections such as bacterial, fungal, protozoan and viral infections; pain; eating disorders, such as obesity, anorexia, and bulimia; asthma; urinary retention; osteoporosis; allergies; Huntington's disease or Gilles dela Tourett's syndrome through detection of mutation in the Human GPR14 gene by the methods described.

In addition, ischemic coronary artery disease (angina and myocardial infarction); atherosclerosis; metabolic diseases (e.g. diabetes); CHF/myocardial dysfunction; arrhythmias; restenosis; hypertension; hypotension; pulmonary disease (hypertension, COPD, asthma); fibrotic vasculopathies (diabetes, SLE, AS, Reynaud's); cerebrovascular events (e.g.

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hemorrhagic and ischemic stroke); neurogenic inflammation/migraine; hematopoietic disorders; ARDS; cancer; autoimmune diseases (e.g. HIV-1 and -2 infection and AIDS); gastrointestinal and genitourinary disturbances (e.g. ulcers); endocrine disorders; fibroproliferative disorders (e.g. psoriasis); inflammatory disease (e.g. RA, Crohn's, IBS);
5 benign prostatic hypertrophy; renal failure and glomerulopathies; disease states, both cardiovascular and non-cardiovascular, which are characterized by excessive vasoconstriction, myocardial dysfunction and/or aberrant fibroproliferative/inflammatory responses; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation, Parkinson's disease, and
10 dyskinesias, infections such as bacterial, fungal, protozoan and viral infections; pain; eating disorders, such as obesity, anorexia, and bulimia; asthma; urinary retention; osteoporosis; allergies; Huntington's disease or Gilles dela Tourett's syndrome, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of Human GPR14 polypeptide or Human GPR14 mRNA.
15 Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an Human GPR14, in a sample derived from a host are well-known to those of skill in the art.
20 Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular 25 location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick,
30 Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to

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the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the
5 affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for
10 the Human GPR14 polypeptides. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the Human GPR14 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal,
15 preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor
et al., *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*,
20 MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss,
Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No.
4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this
invention. Also, transgenic mice, or other organisms including other mammals, may be
25 used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against Human GPR14 polypeptides may also be employed to treat
ischemic coronary artery disease (angina and myocardial infarction); atherosclerosis;
30 metabolic diseases (e.g. diabetes); CHF/myocardial dysfunction; arrhythmias; restenosis;

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hypertension; hypotension; pulmonary disease (hypertension, COPD, asthma); fibrotic vasculopathies (diabetes, SLE, AS, Reynaud's); cerebrovascular events (e.g. hemorrhagic and ischemic stroke); neurogenic inflammation/migraine; hematopoietic disorders; ARDS; cancer; autoimmune diseases (e.g. HIV-1 and -2 infection and AIDS); gastrointestinal and genitourinary disturbances (e.g. ulcers); endocrine disorders; fibroproliferative disorders (e.g. psoriasis); inflammatory disease (e.g. RA, Crohn's, IBS); benign prostatic hypertrophy; renal failure and glomerulopathies; disease states, both cardiovascular and non-cardiovascular, which are characterized by excessive vasoconstriction, myocardial dysfunction and/or aberrant fibroproliferative/inflammatory responses; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation, Parkinson's disease, and dyskinesias, infections such as bacterial, fungal, protozoan and viral infections; pain; eating disorders, such as obesity, anorexia, and bulimia; asthma; urinary retention; osteoporosis; allergies; Huntington's disease or Gilles dela Tourett's syndrome, among others.

15 Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with Human GPR14 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from ischemic coronary artery disease (angina and myocardial infarction); atherosclerosis; metabolic diseases (e.g. diabetes); CHF/myocardial dysfunction; arrhythmias; restenosis; hypertension; hypotension; pulmonary disease (hypertension, COPD, asthma); fibrotic vasculopathies (diabetes, SLE, AS, Reynaud's); cerebrovascular events (e.g. hemorrhagic and ischemic stroke); neurogenic inflammation/migraine; hematopoietic disorders; ARDS; cancer; autoimmune diseases (e.g. HIV-1 and -2 infection and AIDS); gastrointestinal and genitourinary disturbances (e.g. ulcers); endocrine disorders; fibroproliferative disorders (e.g. psoriasis); inflammatory disease (e.g. RA, Crohn's, IBS); benign prostatic hypertrophy; renal failure and glomerulopathies; disease states, both cardiovascular and non-cardiovascular, which are characterized by excessive vasoconstriction, myocardial dysfunction and/or aberrant fibroproliferative/inflammatory responses; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation,

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Parkinson's disease, and dyskinesias, infections such as bacterial, fungal, protozoan and viral infections; pain; eating disorders, such as obesity, anorexia, and bulimia; asthma; urinary retention; osteoporosis; allergies; Huntington's disease or Gilles dela Tourett's syndrome, among others. Yet another aspect of the invention relates to a method of
5 inducing immunological response in a mammal which comprises, delivering Human GPR14 gene via a vector directing expression of Human GPR14 polypeptide in vivo in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation
10 (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a Human GPR14 polypeptide wherein the composition comprises a Human GPR14 polypeptide or Human GPR14 gene. The vaccine formulation may further comprise a suitable carrier. Since Human GPR14 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous,
15 intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations
20 may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the
25 specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

A Human GPR14 polypeptide (receptor of the present invention) may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention. Thus,
30 polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and

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natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

In general, such screening procedures involve providing appropriate cells which
5 express a receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. In particular, a polynucleotide encoding the receptor of the present invention is employed to transfect cells to thereby express a Human GPR14 polypeptide. The expressed receptor is then contacted with a test compound to observe binding, stimulation or inhibition of a functional response.

10 One such screening procedure involves the use of melanophores which are transfected to express a Human GPR14 polypeptide. Such a screening technique is described in PCT WO 92/01810, published February 6, 1992. Such an assay may be employed to screen for a compound which inhibits activation of a receptor of the present invention by contacting the melanophore cells which encode the receptor with both a
15 receptor ligand, such as human or fish urotensin II, and a compound to be screened. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor, *i.e.*, inhibits activation of the receptor.

The technique may also be employed for screening of compounds which activate
20 a receptor of the present invention by contacting such cells with compounds to be screened and determining whether such compound generates a signal, *i.e.*, activates the receptor.

Other screening techniques include the use of cells which express a Human GPR14 polypeptide (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation. In this technique, compounds
25 may be contacted with cells expressing a receptor polypeptide of the present invention. A second messenger response, *e.g.*, signal transduction or pH changes, is then measured to determine whether the potential compound activates or inhibits the receptor.

Another screening technique involves expressing a Human GPR14 polypeptide in which the receptor is linked to phospholipase C or D. Representative examples of such
30 cells include, but are not limited to, endothelial cells, smooth muscle cells, and

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embryonic kidney cells. The screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase second signal.

Another method involves screening for compounds which are antagonists, and thus inhibit activation of a receptor polypeptide of the present invention by determining inhibition of binding of labeled ligand, such as fish or human urotensin II, to cells which have the receptor on the surface thereof, or cell membranes containing the receptor. Such a method involves transfecting a eukaryotic cell with a DNA encoding a Human GPR14 polypeptide such that the cell expresses the receptor on its surface. The cell is then contacted with a potential antagonist in the presence of a labeled form of a ligand, such as fish or human urotensin II. The ligand can be labeled, *e.g.*, by radioactivity. The amount of labeled ligand bound to the receptors is measured, *e.g.*, by measuring radioactivity associated with transfected cells or membrane from these cells. If the compound binds to the receptor, the binding of labeled ligand to the receptor is inhibited as determined by a reduction of labeled ligand which binds to the receptors. This method is called binding assay.

Another such screening procedure involves the use of mammalian cells which are transfected to express a receptor of the present invention. The cells are loaded with an indicator dye that produces a fluorescent signal when bound to calcium, and the cells are contacted with a test substance and a receptor agonist, such as fish or human urotensin II. Any change in fluorescent signal is measured over a defined period of time using, for example, a fluorescence spectrophotometer or a fluorescence imaging plate reader. A change in the fluorescence signal pattern generated by the ligand indicates that a compound is a potential antagonist (or agonist) for the receptor.

Another such screening procedure involves use of mammalian cells which are transfected to express a receptor of the present invention, and which are also transfected with a reporter gene construct that is coupled to activation of the receptor (for example, luciferase or beta-galactosidase behind an appropriate promoter). The cells are contacted with a test substance and a receptor agonist, such as fish or human urotensin II, and the signal produced by the reporter gene is measured after a defined period of time. The signal can be measured using a luminometer, spectrophotometer, fluorimeter, or other

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such instrument appropriate for the specific reporter construct used. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor.

Another such screening technique for antagonists or agonists involves introducing 5 RNA encoding a Human GPR14 polypeptide into *Xenopus* oocytes to transiently or stably express the receptor. The receptor oocytes are then contacted with a receptor ligand, such as human or fish urotensin II, and a compound to be screened. Inhibition or activation of the receptor is then determined by detection of a signal, such as, cAMP, calcium, proton, or other ions.

10 Another method involves screening for a Human GPR14 polypeptide inhibitors by determining inhibition or stimulation of Human GPR14 polypeptide-mediated cAMP and/or adenylate cyclase accumulation or diminution. Such a method involves transiently or stably transfecting a eukaryotic cell with a Human GPR14 polypeptide to express the receptor on the cell surface. The cell is then exposed to potential antagonists in the 15 presence of Human GPR14 polypeptide ligand, such as fish or human urotensin II. The amount of cAMP accumulation is then measured, for example, by radio-immuno or protein binding assays (for example using Flashplates or a scintillation proximity assay). Changes in cAMP levels can also be determined by directly measuring the activity of the enzyme, adenylyl cyclase, in broken cell preparations. If the potential antagonist binds the 20 receptor, and thus inhibits Human GPR14 polypeptide binding, the levels of Human GPR14 polypeptide-mediated cAMP, or adenylate cyclase activity, will be reduced or increased.

Another screening method for agonists and antagonists relies on the endogenous 25 pheromone response pathway in the yeast, *Saccharomyces cerevisiae*. Heterothallic strains of yeast can exist in two mitotically stable haploid mating types, *MATa* and *MATα*. Each cell type secretes a small peptide hormone that binds to a G-protein coupled receptor on opposite mating-type cells which triggers a MAP kinase cascade leading to G1 arrest as a prelude to cell fusion. Genetic alteration of certain genes in the pheromone response 30 pathway can alter the normal response to pheromone, and heterologous expression and coupling of human G-protein coupled receptors and humanized G-protein subunits in yeast cells devoid of endogenous pheromone receptors can be linked to downstream

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signaling pathways and reporter genes (e.g., U.S. Patents 5,063,154; 5,482,835; 5,691,188). Such genetic alterations include, but are not limited to, (i) deletion of the *STE2* or *STE3* gene encoding the endogenous G-protein coupled pheromone receptors; (ii) deletion of the *FAR1* gene encoding a protein that normally associates with cyclin-dependent kinases leading to cell cycle arrest; and (iii) construction of reporter genes fused to the *FUS1* gene promoter (where *FUS1* encodes a membrane-anchored glycoprotein required for cell fusion). Downstream reporter genes can permit either a positive growth selection (e.g., histidine prototrophy using the *FUS1-HIS3* reporter), or a colorimetric, fluorimetric or spectrophotometric readout, depending on the specific reporter construct used (e.g., β -galactosidase induction using a *FUS1-LacZ* reporter).

The yeast cells can be further engineered to express and secrete small peptides from random peptide libraries, some of which can permit autocrine activation of heterologously expressed human (or mammalian) G-protein coupled receptors (Broach, J.R. and Thorner, J. *Nature* 384: 14-16, 1996; Manfredi et al., *Mol. Cell. Biol.* 16: 4700-4709, 1996). This provides a rapid direct growth selection (e.g., using the *FUS1-HIS3* reporter) for surrogate peptide agonists that activate characterized or orphan receptors. Alternatively, yeast cells that functionally express human (or mammalian) G-protein coupled receptors linked to a reporter gene readout (e.g., *FUS1-LacZ*) can be used as a platform for high-throughput screening of known ligands, fractions of biological extracts and libraries of chemical compounds for either natural or surrogate ligands. Functional agonists of sufficient potency (whether natural or surrogate) can be used as screening tools in yeast cell-based assays for identifying G-protein coupled receptor antagonists. For this purpose, the yeast system offers advantages over mammalian expression systems due to its ease of utility and null receptor background (lack of endogenous G-protein coupled receptors) which often interferes with the ability to identify agonists or antagonists.

The present invention also provides a method for determining whether a ligand not known to be capable of binding to a Human GPR14 polypeptide can bind to such receptor which comprises contacting a mammalian cell which expresses a Human GPR14 polypeptide with the ligand, such as fish or urotensin II, under conditions permitting

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binding of candidate ligands to a Human GPR14 polypeptide, and detecting the presence of a candidate ligand which binds to the receptor thereby determining whether the ligand binds to the Human GPR14 polypeptide. The systems hereinabove described for determining agonists and/or antagonists may also be employed for determining ligands
5 which bind to the receptor.

Applicants further discovered that fish or human urotensin II binds equally well to rat GPR14 receptor. Thus all the above described assay techniques can be used for finding agonists and antagonists to rat GPR 14 receptor by employing fish or human urotensinII as a ligand; furthermore, such identified agonists and antagonists are believed
10 to be also agonists and antagonists to Human GPR14 polypeptides. Further aspect of present invention relates to a method of screening for agonists and antagonists of Human GPR14 polypeptides comprising screening for agonists and antagonist for rat GPR14, preferably in the presence of fish or human urotensin II.

Examples of potential Human GPR14 polypeptide antagonists include antibodies
15 or, in some cases, oligonucleotides, which bind to the receptor but do not elicit a second messenger response such that the activity of the receptor is prevented.

Potential antagonists also include proteins which are closely related to a ligand of the Human GPR14 polypeptide, *i.e.* a fragment of the ligand, which have lost biological function and when binding to the Human GPR14 polypeptide, elicit no response.

20 Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, and ligands for Human GPR14 polypeptides, which comprises:

- (a) rat GPR14 or a Human GPR14 polypeptide, preferably that of SEQ ID NO:2, and preferably further comprises labeled or unlabeled fish or human urotensin II;
- 25 (b) a recombinant cell expressing rat GPR14 or a Human GPR14 polypeptide, preferably that of SEQ ID NO:2, and preferably further comprises labeled or unlabeled fish or human urotensin II; or
- (c) a cell membrane expressing rat GPR14 or a Human GPR14 polypeptide; preferably that of SEQ ID NO: 2, and preferably further comprises labeled or unlabeled fish or
30 human urotensin II.

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It will be appreciated that in any such kit, (a), (b), or (c) may comprise a substantial component.

A potential antagonist also includes an antisense construct prepared through the use of antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both methods of which are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix -*see* Lee, *et al.* *Nucl. Acids Res.*, 6: 3073 (1979); Cooney, *et al.*, *Science*, 241: 456 (1988); and Dervan, *et al.*, *Science*, 251: 1360 (1991)), thereby preventing transcription and production of a Human GPR14 polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule to a Human GPR14 polypeptide (antisense - Okano, J., *Neurochem.*, 56: 560 (1991); OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of a Human GPR14 polypeptide.

Another potential antagonist is a small molecule which binds to a Human GPR14 polypeptide, making it inaccessible to ligands such that normal biological activity is prevented. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules.

Potential antagonists also include soluble forms of a Human GPR14 polypeptide, e.g., fragments of the polypeptide, which bind to the ligand and prevent the ligand from interacting with membrane bound Human GPR14 polypeptides.

Human GPR14 proteins are ubiquitous in the mammalian host and are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate Human GPR14 on the one hand and which can inhibit the function of Human GPR14 on the other hand. In general, agonists are

employed for therapeutic and prophylactic purposes for such conditions as ischemic coronary artery disease (angina and myocardial infarction); atherosclerosis; metabolic diseases (e.g. diabetes); CHF/myocardial dysfunction; arrhythmias; restenosis; hypertension; hypotension; pulmonary disease (hypertension, COPD, asthma); fibrotic vasculopathies (diabetes, SLE, AS, Reynaud's); cerebrovascular events (e.g. hemorrhagic and ischemic stroke); neurogenic inflammation/migraine; hematopoietic disorders; ARDS; cancer; autoimmune diseases (e.g. HIV-1 and -2 infection and AIDS); gastrointestinal and genitourinary disturbances (e.g. ulcers); endocrine disorders; fibroproliferative disorders (e.g. psoriasis); inflammatory disease (e.g. RA, Crohn's, IBS); benign prostatic hypertrophy; renal failure and glomerulopathies; disease states, both cardiovascular and non-cardiovascular, which are characterized by excessive vasoconstriction, myocardial dysfunction and/or aberrant fibroproliferative/inflammatory responses; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation, Parkinson's disease, and dyskinesias, infections such as bacterial, fungal, protozoan and viral infections; pain; eating disorders, such as obesity, anorexia, and bulimia; asthma; urinary retention; osteoporosis; allergies; Huntington's disease or Gilles de la Tourette's syndrome.

Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as ischemic coronary artery disease (angina and myocardial infarction); atherosclerosis; metabolic diseases (e.g. diabetes); CHF/myocardial dysfunction; arrhythmias; restenosis; hypertension; hypotension; pulmonary disease (hypertension, COPD, asthma); fibrotic vasculopathies (diabetes, SLE, AS, Reynaud's); cerebrovascular events (e.g. hemorrhagic and ischemic stroke); neurogenic inflammation/migraine; hematopoietic disorders; ARDS; cancer; autoimmune diseases (e.g. HIV-1 and -2 infection and AIDS); gastrointestinal and genitourinary disturbances (e.g. ulcers); endocrine disorders; fibroproliferative disorders (e.g. psoriasis); inflammatory disease (e.g. RA, Crohn's, IBS); benign prostatic hypertrophy; renal failure and glomerulopathies; disease states, both cardiovascular and non-cardiovascular, which are characterized by excessive vasoconstriction, myocardial dysfunction and/or aberrant fibroproliferative/inflammatory responses; psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation,

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Parkinson's disease, and dyskinesias, infections such as bacterial, fungal, protozoan and viral infections; pain; eating disorders, such as obesity, anorexia, and bulimia; asthma; urinary retention; osteoporosis; allergies; Huntington's disease or Gilles dela Tourett's syndrome.

5 Prophylactic and Therapeutic Methods

This invention provides methods of treating an abnormal conditions related to both an excess of and insufficient amounts of Human GPR14 activity.

If the activity of Human GPR14 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the Human GPR14, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

In another approach, soluble forms of Human GPR14 polypeptides still capable of binding the ligand in competition with endogenous Human GPR14 may be administered.

15 Typical embodiments of such competitors comprise fragments of the Human GPR14 polypeptide.

In still another approach, expression of the gene encoding endogenous Human GPR14 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

25 For treating abnormal conditions related to an under-expression of Human GPR14 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates Human GPR14, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy

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may be employed to effect the endogenous production of Human GPR14 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced
5 with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see
10 Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Formulation and Administration

Peptides, such as the soluble form of Human GPR14 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable
15 pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and
20 kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions
25 include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible.

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Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 $\mu\text{g}/\text{kg}$ of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1

A 1.2 kb PCR fragment corresponding to the entire coding region of the *Rattus norvegicus* orphan receptor (GPR14, A. Marchese et al. Genomics, 29(2): 335-44, 1995) was used as a probe to screen a total of .75M plaques from a Human Genomic Placenta library (Stratagene, LaJolla CA, Cat. # 946206). The genomic library screening procedure is described by Elgin, et al. Stratagies 4: 8-9, 1991. The probes were α -32P labeled, using Random Primed Labeling Kit (Boheringer Manheim, Germany, Cat. # 1585584) and purified by running over Sephadex G-50 columns (Pharmacia Biotech. Cat. # 17-0855-02). The hybridization and washing conditions were according to J. Sambrook, E.F. Fritch and T. Maniatis (1989) A Laboratory Manual Second. Ed. Vol. 1 pp. 2.69-2.81 Cold Spring

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Harbor Laboratory Press, Cold Spring Harbor, New York). One Positive phage clone obtained from above screen was further purified. Southern analysis as carried out on digested phage DNA and a 9 kb Sac I fragment hybridized for this clone. This fragment was subcloned into pBlueScript KS vector, and further digested with EcoRI and southern analyzed. A smaller 5kb fragment hybridized to the rat GPR14 probe mentioned above was subcloned into pBlueScript for sequence analysis, the sequence was determined by automated sequencer. A total of 2126 bp were sequenced, this includes an open reading frame encoding a peptide of 390 residues. This sequence is highly homologous to Rat GPR14, as shown by sequence comparisons and also by fasta analysis against the Genbank nucleotide data base.

Example 2: Mammalian Cell Expression

The receptors of the present invention are expressed in either human embryonic kidney 293 (HEK293) cells or adherent dhfr CHO cells. To maximize receptor expression, typically all 5' and 3' untranslated regions (UTRs) are removed from the receptor cDNA prior to insertion into a pCDN or pCDNA3 vector. The cells are transfected with individual receptor cDNAs by lipofectin and selected in the presence of 400 mg/ml G418. After 3 weeks of selection, individual clones are picked and expanded for further analysis. HEK293 or CHO cells transfected with the vector alone serve as negative controls. To isolate cell lines stably expressing the individual receptors, about 24 clones are typically selected and analyzed by Northern blot analysis. Receptor mRNAs are generally detectable in about 50% of the G418-resistant clones analyzed.

Example 3: Ligand bank for binding and functional assays.

A bank of over 200 putative receptor ligands has been assembled for screening. The bank comprises: transmitters, hormones and chemokines known to act via a human seven transmembrane (7TM) receptor; naturally occurring compounds which may be putative agonists for a human 7TM receptor, non-mammalian, biologically active peptides for which a mammalian counterpart has not yet been identified; and compounds not found in nature, but which activate 7TM receptors with unknown natural ligands. This bank is used to initially screen the receptor for known ligands, using both functional (i.e. calcium, cAMP, microphysiometer, oocyte electrophysiology, etc, see below) as well as binding assays.

Example 4: Ligand Binding Assays

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for a receptor is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards its receptor. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell receptor sources. For these assays, specific receptor binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

Example 5: Functional Assay in Xenopus Oocytes

Capped RNA transcripts from linearized plasmid templates encoding the receptor cDNAs of the invention are synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual Xenopus oocytes in response to agonist exposure.. Recordings are made in Ca²⁺ free Barth's medium at room temperature. The Xenopus system can be used to screen known ligands and tissue/cell extracts for activating ligands.

Example 6: Microphysiometric Assays

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, CA). The CYTOSENSOR is thus capable of detecting the activation of a receptor which is coupled to an energy utilizing intracellular signaling pathway such as the G-protein coupled receptor of the present invention.

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Example 7: Extract/Cell Supernatant Screening

A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the 7TM receptor of the invention is also functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify natural ligands. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

Example 8: Calcium and cAMP Functional Assays

7TM receptors which are expressed in HEK 293 cells have been shown to be coupled functionally to activation of PLC and calcium mobilization and/or cAMP stimulation or inhibition. Basal calcium levels in the HEK 293 cells in receptor-transfected or vector control cells were observed to be in the normal, 100 nM to 200 nM, range. HEK 293 cells expressing recombinant receptors are loaded with fura 2 and in a single day > 150 selected ligands or tissue/cell extracts are evaluated for agonist induced calcium mobilization. Similarly, HEK 293 cells expressing recombinant receptors are evaluated for the stimulation or inhibition of cAMP production using standard cAMP quantitation assays. Agonists presenting a calcium transient or cAMP fluctuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing receptor.

Example 9: Urotensin II-induced Ca²⁺ mobilization in transient HEK 293 GPR14 and stable HEK 293 G?16 GPR14 cells.

The GPR14 receptor when transiently transfected into HEK 293 cells responds to urotensin II in a concentration-dependent manner with a robust calcium response. Both the transiently transfected rat and human receptors respond to fish urotensin II with similar affinities. Figure 3 shows the concentration response curves for fish urotensin II against both the rat and human receptors. The data was generated with the 96 well FLuorescent Imaging Plate Reader (FLIPR). Each point is the mean of 6-8 individual wells in 3-4 separate 96 well plates read on FLIPR. The EC₅₀ for fish urotensin II was 0.5 nM for the human receptor and 0.8 nM for the rat receptor.

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Additionally the human receptor was stably transfected into HEK 293 G?16 cells and human urotensin II produced concentration dependent calcium mobilization responses. Figure 4 is the concentration-response-curves for human urotensin II for 3 clones (designated L2, L3 and L4) of the stably transfected cells. The EC50s of human urotensin II for the 3
5 clones were 0.1-0.2 nM.

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What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the polypeptide of SEQ ID NO:2 or
5 the corresponding fragment thereof; or a nucleotide sequence complementary to said nucleotide sequence.

2. The polynucleotide of claim 1 which is DNA or RNA.

10 3. The polynucleotide of claim 1 wherein said nucleotide sequence is at least 80% identical to that contained in SEQ ID NO:1.

15 4. The polynucleotide of claim 3 wherein said nucleotide sequence is contained in SEQ ID NO:1.

5. The polynucleotide of claim 1 wherein said encoding nucleotide sequence encodes the polypeptide of SEQ ID NO:2 or a fragment thereof.

20 6. A polynucleotide probe or primer comprising at least 15 contiguous nucleotides of the polynucleotide of claim 3.

7. A DNA or RNA molecule comprising an expression system wherein said expression system is capable of producing a Human GPR14 or a fragment thereof having at least 80% identity with a nucleotide sequence encoding the polypeptide of SEQ ID
25 NO:2 or said fragment when said expression system is present in a compatible host cell.

8. A host cell comprising the expression system of claim 7.

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9. A process for producing a Human GPR14 polypeptide or fragment comprising culturing a host of claim 8 and under conditions sufficient for the production of said polypeptide or fragment.

5

10. The process of claim 9 wherein said polypeptide or fragment is expressed at the surface of said cell.

11. Cells produced by the process of claim 10.

10

12. The process of claim 9 which further includes recovering the polypeptide or fragment from the culture.

15. 13. A process for producing a cell which produces a Human GPR14 polypeptide or a fragment thereof comprising transforming or transfecting a host cell with the expression system of claim 7 such that the host cell, under appropriate culture conditions, produces a Human GPR14 polypeptide or fragment.

20. 14. A Human GPR14 polypeptide or a fragment thereof comprising an amino acid sequence which is at least 85 % identical to the amino acid sequence contained in SEQ ID NO:2.

25. 15. The polypeptide of claim 14 which comprises the amino acid sequence of SEQ ID NO:2, or a fragment thereof.

16. A Human GPR14 polypeptide or fragment prepared by the method of claim 12.

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17. An antibody immunospecific for the Human GPR14 polypeptide of claim
14.

5 18. A method for the treatment of a subject in need of enhanced Human GPR14
activity comprising:

- (a) administering to the subject a therapeutically effective amount of an agonist
to said receptor; and/or
- (b) providing to the subject Human GPR14 polynucleotide in a form so as to
10 effect production of said receptor activity *in vivo*.

19. A method for the treatment of a subject having need to inhibit Human
GPR14 activity comprising:

- (a) administering to the subject a therapeutically effective amount of an
15 antagonist to said receptor; and/or
- (b) administering to the subject a nucleic acid molecule that inhibits the
expression of the nucleotide sequence encoding said receptor; and/or
- (c) administering to the subject a therapeutically effective amount of a
polypeptide that competes with said receptor for its ligand.

20

20. A process for diagnosing a disease or a susceptibility to a disease in a
subject related to expression or activity of Human GPR14 in a subject comprising:

- (a) determining the presence or absence of a mutation in the nucleotide
sequence encoding said Human GPR14 in the genome of said subject; and/or
- 25 (b) analyzing for the presence or amount of the Human GPR14 expression in a
sample derived from said subject.

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21. A method for identifying compounds which bind to Human GPR14 comprising:

- (a) contacting cells of claim 11 with a candidate compound; and
- (b) assessing the ability of said candidate compound to bind to said cells.

5

22. The method of claim 21 which further includes determining whether the candidate compound effects a signal generated by activation of the Human GPR14 polypeptide at the surface of the cell, wherein a candidate compound which effects production of said signal is identified as an agonist.

10

23. A method for identifying agonist or antagonist of a Human GPR14 polypeptide which comprises:

contacting a cell expressing on the surface thereof the polypeptide, said polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and

determining whether the compound binds to and activates or inhibits the polypeptide by measuring the level of a signal generated from the interaction of the compound with the polypeptide.

20

24. A method of claim 23 which further comprises conducting the identification of agonist or antagonist in the presence of labeled or unlabeled, fish or human urotensin II.

25

25. A method for identifying agonist or antagonist of a Human GPR14 polypeptide which comprises:

determining the inhibition of binding of a ligand to cells which have the polypeptide on the surface thereof, or to cell membranes containing the polypeptide, in

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the presence of a candidate compound under conditions to permit binding to the polypeptide, and determining the amount of ligand bound to the polypeptide, such that a compound capable of causing reduction of binding of a ligand is an agonist or antagonist.

5 26. A method of claim 25 in which a ligand is labeled or unlabeled, fish or human urotensin II.

27. A method for identifying agonist or antagonist of rat GPR14 receptor polypeptide which comprises:

10 contacting a cell expressing on the surface thereof the polypeptide, said polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and

15 determining whether the compound binds to and activates or inhibits the polypeptide by measuring the level a signal generated from the interaction of the compound with the polypeptide.

20 28. A method of claim 27 which further comprises conducting the identification of agonist or antagonist in the presence of labeled or unlabeled, fish or human urotensin II.

29. A method for identifying agonist or antagonist of rat GPR14 receptor polypeptide which comprises:

25 determining the inhibition of binding of a ligand to cells which have the polypeptide on the surface thereof, or to cell membranes containing the polypeptide, in the presence of a candidate compound under conditions to permit binding to the polypeptide, and determining the amount of ligand bound to the polypeptide, such that a compound capable of causing reduction of binding of a ligand is an agonist or antagonist.

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30. A method of claim 29 in which a ligand is labeled or unlabeled, fish or human urotensin II.

5 31. An agonist identified by the method of claim 22.

32. The method of claim 21 which further includes contacting said cell with a known agonist for said Human GPR14; and

10 determining whether the signal generated by said agonist is diminished in the presence of said candidate compound, wherein a candidate compound which effects a diminution in said signal is identified as an antagonist for said Human GPR14.

33. An antagonist identified by the method of claim 24.

Nucleotide and Amino Acid sequence of Human GPR14 (SEQ ID NOS: 1 and 2, respectively.)

10	30	50
GACAAAAC	TGGGTACGGCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATCGAAT	
70	90	110
TCGTTTCCCTGTATGAGAAATGGAGATTGCAGAGGCCTCCTCTCCTTACATGTTCTTCT		
130	150	170
ATTTGGACTTTAAAGTCAGTAGCTACTAGTTGCAATCTAAAGAAAACATTTTTAA		
190	210	230
ATGTACAAGTCAAATAAACGAgAAAGGACTCAGGAGTAAGTGGGCCACCTGTGCAC		
250	270	290
AGACAAgAAAGTGAGGCCTGGGGGGCGCACTGGGCAgAgCCCAGGACTCCCAGTTCTGT		
310	330	350
CCACTGCCGACCTCTGCCCAAGGGCTGCCCTCCTGTGTTCCGGCTTCAgAAAAGCCA		
370	390	410
GTTCATCCCAGAgGCCATGGGACCTACAgTGAgGGGGGGCAgGGGTCTGCTGGGCAT		
430	450	470
GCGGGGGTCgGGGAgGGGGTTGGGCAgCTCGTCTGGCTCTGAGTCCTCCTGCAG		
490	510	530
AgCTGGTGGCTTCAgAgAGTCCCAGAgAGTTGGAGGGCACTGGGAgCCCACGTGACTCT		
550	570	590
GTGGGAACAgGCCATCACAGTGGCCTCCTGGAgCGGAAgGTGTTGCCTGATTTGCTTC		
610	630	650
TTTCCCCACAGGCTGAgCTGGTTGCCACAGGGGCCCGCCCCATCTCAgGGAgTGTCC		
670	690	710
ACCCAgCCCTGAgCCCGTCgTGAGGGTCAgAgATGGCGCTGACCCCCgAGTCCCCGAGC		
	M A L T P E S P S	
730	750	770
AGCTTCCCTGGGCTGGCCGCCACCGGCAGCTCTGTGCCGGAGCCGCCCTGGCGGCCAAC		
S F P G L A A T G S S V P E P P G G P N		
790	810	830
GCAACCCTCAACAGCTCCTGGGCCAGCCGACCGAGCCAGCTCCCTGGAgGACCTGGTG		
A T L N S S W A S P T E P S S L E D L V		
850	870	890

FIG. 1

GCCACGGGCACCATTGGGACTCTGCTGTCGCCATGGCGTGGTGGCGTGGTGGCAAC
 A T G T I G T L L S A M G V V G V V G N
 910 930 950
 GCCTACACGCTGGTGGTCACCTGCCGCTCCCTGCGTGCCTGGCCTCCATGTACGTCTAC
 A Y T L V V T C R S L R A V A S M Y V Y
 970 990 1010
 GTGGTCAACCTGGCGCTGGCGACCTGCTGTACCTGCTCAGCATCCCCCTCATCGTGGCC
 V V N L A L A D L L Y L L S I P F I V A
 1030 1050 1070
 ACCTACGTCACCAAGGAGTGGCACCTCGGGGAcGTGGGCTGCCGCGTGCCTTCGGCCTG
 T Y V T K E W H F G D V G C R V L F G L
 1090 1110 1130
 GACTTCCTGACCATGCACGCCAGCATCTCACGCTGACCGTCATGAGCAGCGAGCGCTAC
 D F L T M H A S I F T L T V M S S E R Y
 1150 1170 1190
 GCTGCGGTGCTGCGGCCGCTGGACACCGTGAGCGCCCCAAGGGCTACCGCAAGCTGCTG
 A A V L R P L D T V Q R P K G Y R K L L
 1210 1230 1250
 GCGCTGGGCACCTGGCTGCTGGCGCTGCTGACgCTGCCGTGATGCTGGCCATGCGG
 A L G T W L L A L L T L P V M L A M R
 1270 1290 1310
 CTGGTGCGCCGGGTcCAAAGAgCCTGTGCCTGCCGCTGGGGCCCGCGCGCCACCGC
 L V R R G P K S L C L P A W G P R A H R
 1330 1350 1370
 GCCTACCTGACGCTGCTCTCGCCACCAAGCATCGGGGGCCCGGGCTGCTCATCGGCTG
 A Y L T L L F A T S I A G P G L L I G L
 1390 1410 1430
 CTCTACGCGCGCCTGGCCCGCCTACCGCCGCTCGCAGCGCGCCTCCTCAAGCGGGCC
 L Y A R L A R A Y R R S Q R A S F K R A
 1450 1470 1490
 CGGCGGCCGGGGCGCGCGCTGCCCTGGTGCTGGCATCGTGTGCTCTCTGGGCC
 R R P G A R A L R L V L G I V L L F W A
 1510 1530 1550
 TGCTTCCTGCCCTCTGGCTGTGGCAGCTGCTGCCAGTACCAACCAGGCCCGCTGGCG
 C F L P F W L W Q L L A Q Y H Q A P L A
 1570 1590 1610

FIG. 1A

CCGCGGACGGCGCATCGTCAACTACCTGACCACCTGCCTCACCTACGGAACAGCTGC
 P R T A R I V N Y L T T C L T Y G N S C
 1630 1650 1670
 GCCAACCCCTCCTCTACACGCTGCTCACAGGAACCTACCGCGACCACCTGCAGGGCGC
 A N P F L Y T L L T R N Y R D H L R G R
 1690 1710 1730
 GTGCGGGGCCGGGCAGCGGGGAGGCCGGGGCCGTTCCCTCCCTGCAGCCCCGCGCC
 V R G P G S G G G R G P V P S L Q P R A
 1750 1770 1790
 CGCTTCCAGCGCTGTTGGGGCGCTCCCTGTCTCCTGCAGCCCACAGCCCAGCAGC
 R F Q R C S G R S L S S C S P Q P T D S
 1810 1830 1850
 CTCGTGCTGGCCCCAGCGGGCCCGACCTGCAGCCCCAgGGTCCCAGGGCCCCGGCG
 L V L A P A A P A R P A P E G P R A P A
 1870 1890 1910
 TGAGCACGCGGAGGGGGCGGCTGGAGTCCAgGCAGGGACgCGCCCCAAAGCCCCAGCCACT
 *
 1930 1950 1970
 CCCGGGAGCCCCCCCCAACTCCCAATCACAGGCCCTGCCCTCCTCCGTCCCCCTCTGGA
 1990 2010 2030
 AAGATcCTGCTCGCTTCCCTCAgCGCCCTCCGTGATGCCCAgAAgCGCCCAcCCGCC
 2050 2070 2090
 TCCCTGAgGGTcTCCAgGAgGcTCCAgCGCAGTCCGGCTTCTGGAgAcATGGcTTCgtC
 2110
 ACAGAgGGcAgCAGGcGCCATTGCC

FIG. 1B

Amino acid sequence of Rat GPR14

1 MALSLESTTS FHMLTVSGST VTELPGDSNV SLNSSWSGPT DPSSLKDLVA
51 TGVIGAVLSA MGVVGMVGNV YTLVVMCRFL RASASMYVYV VNLALADLLY
101 LLSIPFIIAT YVTKDWHFGD VGCRVLFSLD FLTMHASIFT LTIMSSERYA
151 AVLRLPLDTVQ RSKGYRKLLV LGTWLLALLL TLPMMILAIQL VRRGSKSLCL
201 PAWGPRAHRT YLTLLFGTSI VGPGLVIGLL YVRLARAYWL SQQASFQKTR
251 RLPNPRVLYL ILGIVLLFWA CFLPFWLWQL LAQYHEAMPL TPETARIIVNY
301 LTTCLTYGNS CINPLLYTLL TKNYREYLRG RQRSLGSSCH SPGSPGSFLP
351 SRVHLQQDSG RSLSSSSQQA TETLMLSPVP RNGALL

FIG. 2A

Nucleotide sequence of rat GPR14

1 GGGACAGTGG GTCCCAATGG CTCTAGGGTC CTCCTGTGTA GCTGGGGAGA
51 TAACAAAAAA GGGATTCTTT TGAGGCTTCC AACAGGATAT AGGACCTGGT
101 GAGCCTTTGT CTCTCTGCAT AGGGACAGTG ACTGTGTCCA TCACAGAGGC
151 TGTTTAGGGC ATAGAAGTAG GTTACTGCCT TGAACCTCTG ACACTAATCT
201 TTTCCCACAG GACAAGTTTC CCACGGGCTC TCCTCACTGA GCAGTGGTTC
251 TCCCCCTGGA ATCCCAGTGT GAGGACCGAG ATGGCTCTGA GCCTGGAGTC
301 TACAACAAAGC TTTCATATGC TCACCGTGTC CGGAAGCACT GTGACTGAGC
351 TGCCTGGTGA CTCCAACGTG TCCCTCAACA GTTCCTGGTC CGGCCCAACA
401 GATCCCAGCT CCCTGAAAGA CCTTGTGGCC ACGGGTGTCA TCGGGGCAGT
451 GCTCTCAGCC ATGGGTGTGG TGGGCATGGT GGGAAATGTA TACACTTTGG
501 TGGTCATGTG CCGGTTTCTG CGTGCCTCGG CCTCCATGTA CGTCTATGTG
551 GTCAAACCTAG CGCTGGCTGA TCTGCTGTAC CTGCTGAGCA TTCCCTTCAT
601 CATAGCCACC TACGTCACTA AGGACTGGCA CTTTGGAGAT GTGGGCTGCA
651 GAGTCCTCTT TAGCCTGGAC TTCCTGACAA TGCACGCCAG CATCTTCACC
701 CTGACCATAA TGAGCAGCGA ACGCTATGCA GCCGTACTGA GGCCTCTGGA
751 CACAGTCCAG CGCTCCAAGG GTTACCGTAA GCTGCTGGTG CTGGGCACCT
801 GGTTGCTGGC ACTGCTGCTG ACCCTACCCA TGATGCTTGC CATCCAGCTG
851 GTCCCGCAGGG GCTCTAACAG CCTCTGCCTG CCAGCCTGGG GCCCTCGTGC
901 CCACCGTACT TACCTAACGT TGCTCTTGG GACCAGCATT GTGGGGCCTG

FIG. 2B

951 GCTTGGTCAT TGGGCTGCTC TATGTCCGTC TGGCCAGGGC CTACTGGCTA
1001 TCTCAGCAAG CTTCTTCAA GCAGACACGG CGGCTGCCA ACCCCAGGGT
1051 GCTCTACCTC ATCCTTGGTA TCGTCCTTCT CTTCTGGGCC TGCTTTCTAC
1101 CCTTCTGGCT GTGGCAGCTG CTGGCCCAGT ACCACGAGGC CATGCCACTG
1151 ACTCCCGAGA CTGCACGCAT TGTCAACTAC CTGACCACCT GCCTCACTTA
1201 TGGCAACAGT TGCATCAATC CCTTGCTCTA CACTCTGCTC ACCAAGAACT
1251 ATCGAGAGTA CCTACGTGGC CGCCAGCGGT CACTGGTAG TAGTTGCCAC
1301 AGCCCAGGGA GTCCTGGCAG CTTCCTGCC AGCCGAGTCC ACCTCCAGCA
1351 GGACTCGGGC CGCTCGCTGT CCTCCAGCAG CCAACAGGCC ACAGAGACCC
1401 TCATGCTGTC TCCAGTCCCC CGTAACGGGG CCCTTCTCTG AGAGTGCACT
1451 GTGCAATCCT GGCATAGGAA AGGACCCAAA GGC GTGCCGGC TCCGGAGCGC
1501 ATTTCCCAGA ATCCCCTGCT CAAACCTAAC TGGCTCGTC

FIG. 2B cont'd.

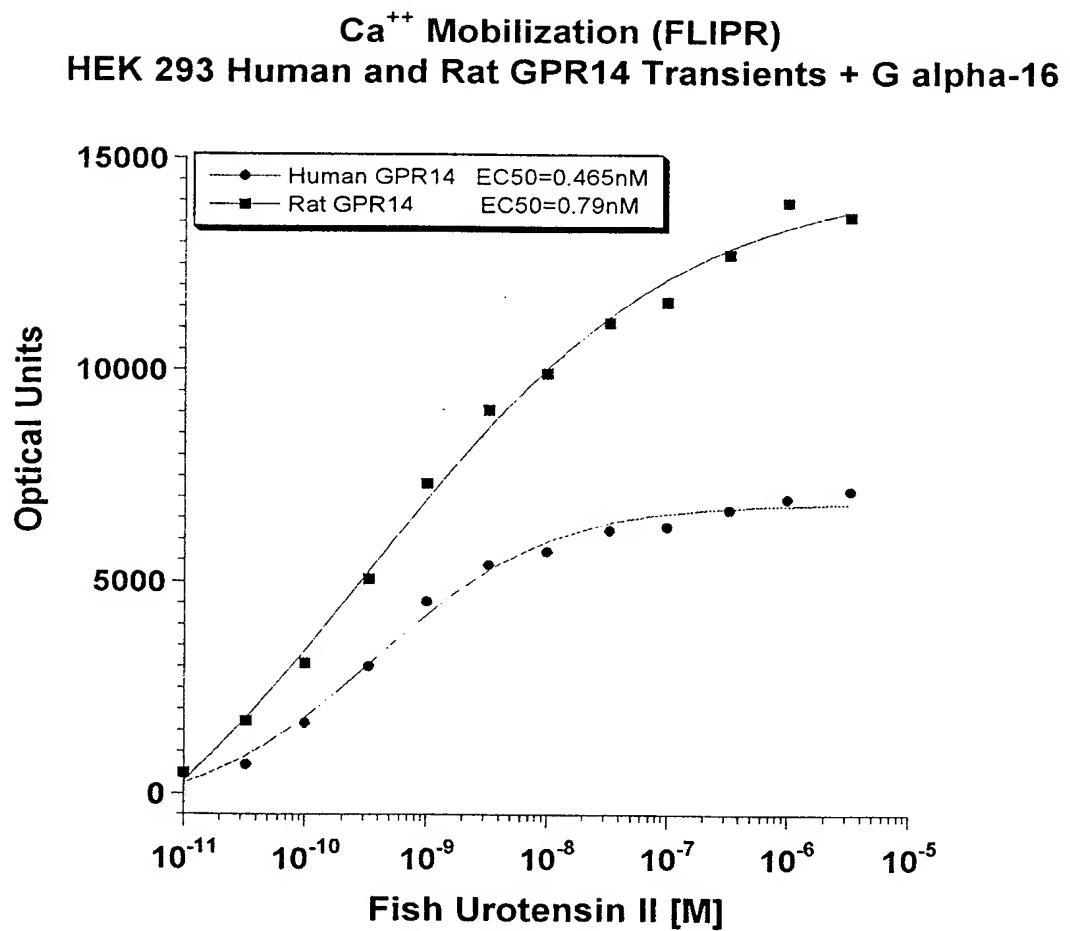


FIG. 3

Ca²⁺ Mobilization (FLIPR)
HEK 293 Galpha16 GPR14 Stable Clones

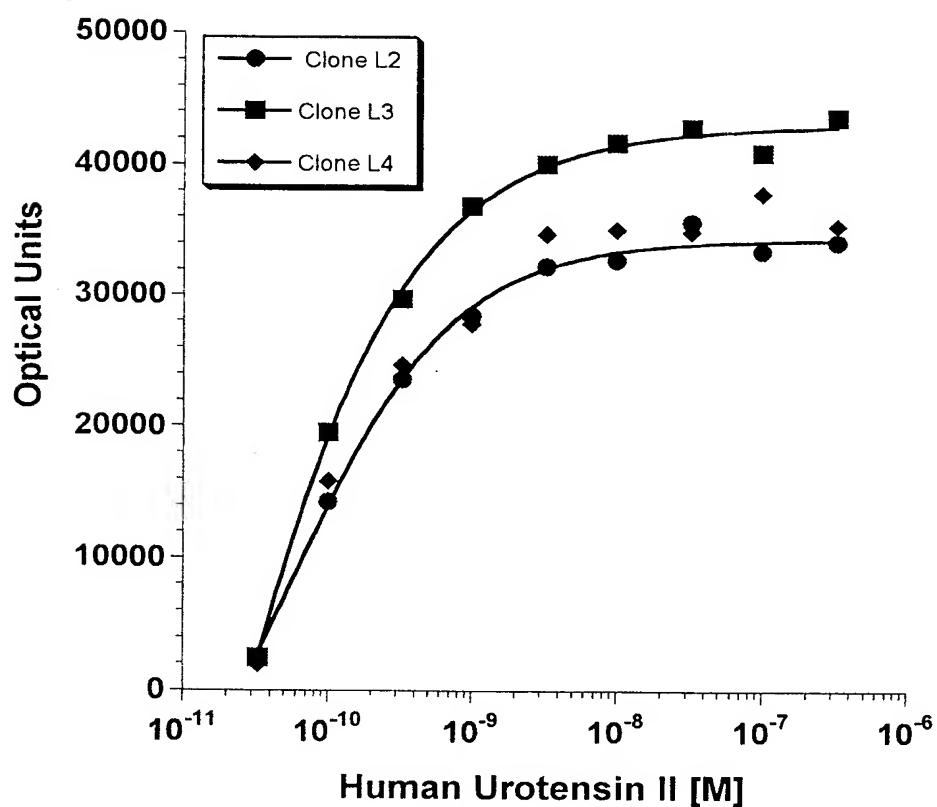


FIG. 4

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SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: SMITHKLINE BEECHAM CORPORATION
SMITHKLINE BEECHAM plc
SMITHKLINE BEECHAM LABORATOIRES PHARMACEUTIQUES

(ii) TITLE OF THE INVENTION: A METHOD OF FINDING AGONIST
AND ANTAGONIST TO HUMAN AND RAT GPR14

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Ratner & Prestia
(B) STREET: P.O. Box 980
(C) CITY: Valley Forge
(D) STATE: PA
(E) COUNTRY: USA
(F) ZIP: 19482

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 27-JAN-1999
(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/789,354
(B) FILING DATE: 27-JAN-1997

(A) APPLICATION NUMBER: 60/074,075
(B) FILING DATE: 09-FEB-1998

(A) APPLICATION NUMBER: 09/058,725
(B) FILING DATE: 10-APR-1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Prestia, Paul F
(B) REGISTRATION NUMBER: 23,031
(C) REFERENCE/DOCKET NUMBER: GP-50005-2

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 610-407-0700
(B) TELEFAX: 610-407-0700
(C) TELEX: 846169

(2) INFORMATION FOR SEQ ID NO:1:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2126 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GACAAA	ACTG	GGTACGGGCC	CCCCTCGAGG	TCGACGGTAT	CGATAAGCTT	GATATCGAAT	60
TCGTTT	CCCT	GTATGAGAAA	TGGAGATTGC	AGAGGCCTTC	CTCTCCTTAC	ATGTTCTTCT	120
ATTTGG	ACTT	TTAAAGTCAG	TAGCTACTAG	TTTGCAATC	TAAAGAAAAC	ATTTTTTAA	180
ATGTACA	AAGT	CAAATAAATA	CGAGAAAGGA	CTCAGGAGTA	AGTGGGCC	ACCTGTGCAC	240
AGACAAG	AAA	GTGAGGCTG	GGGGGGCGCA	CTGGGCAGAG	CCCAGGACTC	CCAGTTCTGT	300
CCACTGCC	GA	CCTCTGCC	AGGGGCTGCC	CTCCTGTGTT	CCGGCTTTCA	GAAAAGCCCA	360
GTTCAT	CCCA	GAGGCCATGG	GACTTACAGT	GAGGGGGGGG	CAGGGGTCTC	GCTGGGGCAT	420
GCGGGG	TGCG	GGGAGGGGGG	TTGGGGCAGC	TCGTCTGGT	GCTCTTGAGT	CCTCTGCAG	480
AGCTGG	TGGC	TTCCAGAGAG	TCCCGAGAGT	TGGAGGGCAC	TGGGGAGCCC	ACGTGACTCT	540
GTGGGA	ACGA	GGCCATCACA	GTGGCCTCCT	GGGAGCGGAA	GGTGTTGCT	GATTGCTTC	600
TTTCCC	CCCA	GGCTGAGCTG	GTTGCCACCA	GGGGCCCCCG	CCCCATCTCA	GGGAGTGTCC	660
ACCCAG	CCCT	GAGCCCGTCG	TGAGGGGTCA	GAGATGGCG	TGACCCCCGA	GTCCCCGAGC	720
AGCTTCCC	TG	GGCTGGCC	CACCGGAGC	TCTGTGCC	AGCCGCTGG	CGGCCCAAC	780
GCAACC	CTCA	ACAGCTCCTG	GGCCAGCCC	ACCGAGCCC	GCTCCCTGGA	GGACCTGGT	840
GCCAC	GGCA	CCATTGGGAC	TCTGCTGTC	GCCATGGCG	TGGTGGCGT	GGTGGCAAC	900
GCCTAC	ACAC	TGGTGGTCAC	CTGCCGCTC	CTGCGTGC	TGGCCTCAT	GTACGTCTAC	960
GTGGTCA	ACC	TGGCGCTGGC	CGACCTGCTG	TACCTGCTA	GCATCCCC	CATCGTGGCC	1020
ACCTAC	GTCA	CCAAGGAGTG	GCAC	GACGTGGC	GGCGCGT	CTTCGGCCTG	1080
GA	CTCTGA	CCATGCAC	CGAC	ACGCTGACCG	TCATGAGCAG	CGAGCGCTAC	1140
GCTGCG	GTG	TGCGGCC	GGACACCGT	CAGGCC	AGGGCTACCG	CAAGCTGCTG	1200
GCGCTGG	CA	CCTGGCTG	GGCGCTG	CTGACGCTG	CCGTGATG	GGCCATGCGG	1260
CTGGT	CGCC	GGGGTCC	GGACCTGT	CTGCCC	GGGGCCCGCG	CGCCACCGC	1320
GCCTAC	CTGA	CGCTGCT	CGCCACCAGC	ATCGCGGG	CCGGGCTG	CATCGGGCTG	1380
CTCTAC	CGC	GCCTGGCC	CGCCTACCG	CGCTCG	GCGCCTC	CAAGCGGGCC	1440
CGGCGG	CCGG	GGGCGCGC	GCTGCGC	GTGCTGG	TCGTGCTG	CTTCTGGCC	1500
TGCTT	CC	CCTTCTGG	GTGGCAGCT	CTCGCC	ACCACCA	CCCGCTGGCG	1560
CCGCGG	ACGG	CGCGCATCG	CAACTAC	ACCAC	TACCTAC	CAACAGCTG	1620
GCCAA	CCCCT	TCCTCTACAC	GCTGCTC	AGGA	ACTACC	GCGACCAC	1680
GTGCGG	GGCC	CGGGCAGCG	GGGAGGCC	GGGCC	GGT	CCCGCCG	1740
CGCTT	CCAGC	GCTGTT	CCGCTCC	TCTT	CCTG	CCCCACAG	1800
CTCGT	GCTG	CCCCAGCG	CCCGGCC	CCTG	CGCC	CACTGAC	1860
TGAGC	ACGCG	GAGGGGCC	TGGAGTCC	GCGGG	GAC	CCCAGCC	1920
CCC	GGGAG	CCCAACTC	CCAAATCACA	GGCC	CTG	CCCTTCTG	1980
AAGAT	CCTG	TCGCTT	TCAGCGCC	TCCCG	TGAT	CCCAGAAG	2040
TCC	CTGAG	TCTCCAGG	GCTCCAGC	AGTCCC	GGCT	TCTGGAGACA	2100
ACAGAG	GGCA	GCAGGCG	TTGCC				2126

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 389 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met	Ala	Leu	Thr	Pro	Glu	Ser	Pro	Ser	Ser	Phe	Pro	Gly	Leu	Ala	Ala
1				5					10					15	
Thr	Gly	Ser	Ser	Val	Pro	Glu	Pro	Pro	Gly	Gly	Pro	Asn	Ala	Thr	Leu
	20				25							30			
Asn	Ser	Ser	Trp	Ala	Ser	Pro	Thr	Glu	Pro	Ser	Ser	Leu	Glu	Asp	Leu
	35					40					45				
Val	Ala	Thr	Gly	Thr	Ile	Gly	Thr	Leu	Leu	Ser	Ala	Met	Gly	Val	Val
	50				55					60					
Gly	Val	Val	Gly	Asn	Ala	Tyr	Thr	Leu	Val	Val	Thr	Cys	Arg	Ser	Leu
	65					70				75			80		
Arg	Ala	Val	Ala	Ser	Met	Tyr	Val	Tyr	Val	Val	Asn	Leu	Ala	Leu	Ala
		85					90					95			
Asp	Leu	Leu	Tyr	Leu	Leu	Ser	Ile	Pro	Phe	Ile	Val	Ala	Thr	Tyr	Val
		100						105				110			
Thr	Lys	Glu	Trp	His	Phe	Gly	Asp	Val	Gly	Cys	Arg	Val	Leu	Phe	Gly
		115					120				125				
Leu	Asp	Phe	Leu	Thr	Met	His	Ala	Ser	Ile	Phe	Thr	Leu	Thr	Val	Met
		130					135				140				
Ser	Ser	Glu	Arg	Tyr	Ala	Ala	Val	Leu	Arg	Pro	Leu	Asp	Thr	Val	Gln
	145					150				155			160		
Arg	Pro	Lys	Gly	Tyr	Arg	Lys	Leu	Leu	Ala	Leu	Gly	Thr	Trp	Leu	Leu
		165					170				175				
Ala	Leu	Leu	Leu	Thr	Leu	Pro	Val	Met	Leu	Ala	Met	Arg	Leu	Val	Arg
		180					185				190				
Arg	Gly	Pro	Lys	Ser	Leu	Cys	Leu	Pro	Ala	Trp	Gly	Pro	Arg	Ala	His
		195					200				205				
Arg	Ala	Tyr	Leu	Thr	Leu	Leu	Phe	Ala	Thr	Ser	Ile	Ala	Gly	Pro	Gly
		210					215				220				
Leu	Leu	Ile	Gly	Leu	Leu	Tyr	Ala	Arg	Leu	Ala	Arg	Ala	Tyr	Arg	Arg
	225				230				235			240			
Ser	Gln	Arg	Ala	Ser	Phe	Lys	Arg	Ala	Arg	Arg	Pro	Gly	Ala	Arg	Ala
		245					250				255				
Leu	Arg	Leu	Val	Leu	Gly	Ile	Val	Leu	Leu	Phe	Trp	Ala	Cys	Phe	Leu
		260					265				270				
Pro	Phe	Trp	Leu	Trp	Gln	Leu	Leu	Ala	Gln	Tyr	His	Gln	Ala	Pro	Leu
		275					280				285				
Ala	Pro	Arg	Thr	Ala	Arg	Ile	Val	Asn	Tyr	Leu	Thr	Thr	Cys	Leu	Thr
		290				295				300					
Tyr	Gly	Asn	Ser	Cys	Ala	Asn	Pro	Phe	Leu	Tyr	Thr	Leu	Leu	Thr	Arg
	305				310				315			320			
Asn	Tyr	Arg	Asp	His	Leu	Arg	Gly	Arg	Val	Arg	Gly	Pro	Gly	Ser	Gly
		325					330				335				
Gly	Gly	Arg	Gly	Pro	Val	Pro	Ser	Leu	Gln	Pro	Arg	Ala	Arg	Phe	Gln
		340					345				350				
Arg	Cys	Ser	Gly	Arg	Ser	Leu	Ser	Ser	Cys	Ser	Pro	Gln	Pro	Thr	Asp
		355					360				365				
Ser	Leu	Val	Leu	Ala	Pro	Ala	Ala	Pro	Ala	Arg	Pro	Ala	Pro	Glu	Gly
		370					375				380				
Pro	Arg	Ala	Pro	Ala											
	385														

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala	Gly	Thr	Ala	Asp	Cys	Phe	Trp	Lys	Tyr	Cys	Val
1			5					10			

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 386 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Leu	Ser	Leu	Glu	Ser	Thr	Thr	Ser	Phe	His	Met	Leu	Thr	Val
1				5				10				15			
Ser	Gly	Ser	Thr	Val	Thr	Glu	Leu	Pro	Gly	Asp	Ser	Asn	Val	Ser	Leu
				20			25					30			
Asn	Ser	Ser	Trp	Ser	Gly	Pro	Thr	Asp	Pro	Ser	Ser	Leu	Lys	Asp	Leu
				35			40					45			
Val	Ala	Thr	Gly	Val	Ile	Gly	Ala	Val	Leu	Ser	Ala	Met	Gly	Val	Val
	50				55			60							
Gly	Met	Val	Gly	Asn	Val	Tyr	Thr	Leu	Val	Val	Met	Cys	Arg	Phe	Leu
	65				70			75				80			
Arg	Ala	Ser	Ala	Ser	Met	Tyr	Val	Tyr	Val	Val	Asn	Leu	Ala	Leu	Ala
					85			90				95			
Asp	Leu	Leu	Tyr	Leu	Leu	Ser	Ile	Pro	Phe	Ile	Ile	Ala	Thr	Tyr	Val
					100			105				110			
Thr	Lys	Asp	Trp	His	Phe	Gly	Asp	Val	Gly	Cys	Arg	Val	Leu	Phe	Ser
				115			120				125				
Leu	Asp	Phe	Leu	Thr	Met	His	Ala	Ser	Ile	Phe	Thr	Leu	Thr	Ile	Met
	130				135			140							
Ser	Ser	Glu	Arg	Tyr	Ala	Ala	Val	Leu	Arg	Pro	Leu	Asp	Thr	Val	Gln
	145				150			155				160			
Arg	Ser	Lys	Gly	Tyr	Arg	Lys	Leu	Leu	Val	Leu	Gly	Thr	Trp	Leu	Leu
					165			170				175			
Ala	Leu	Leu	Leu	Thr	Leu	Pro	Met	Met	Leu	Ala	Ile	Gln	Leu	Val	Arg
					180			185				190			
Arg	Gly	Ser	Lys	Ser	Leu	Cys	Leu	Pro	Ala	Trp	Gly	Pro	Arg	Ala	His
	195				200			205							
Arg	Thr	Tyr	Leu	Thr	Leu	Leu	Phe	Gly	Thr	Ser	Ile	Val	Gly	Pro	Gly
	210				215			220							
Leu	Val	Ile	Gly	Leu	Leu	Tyr	Val	Arg	Leu	Ala	Arg	Ala	Tyr	Trp	Leu
	225				230			235				240			
Ser	Gln	Gln	Ala	Ser	Phe	Lys	Gln	Thr	Arg	Arg	Leu	Pro	Asn	Pro	Arg
					245			250				255			
Val	Leu	Tyr	Leu	Ile	Leu	Gly	Ile	Val	Leu	Leu	Phe	Trp	Ala	Cys	Phe
					260			265				270			
Leu	Pro	Phe	Trp	Leu	Trp	Gln	Leu	Leu	Ala	Gln	Tyr	His	Glu	Ala	Met
					275			280				285			
Pro	Leu	Thr	Pro	Glu	Thr	Ala	Arg	Ile	Val	Asn	Tyr	Leu	Thr	Thr	Cys
	290				295			300							

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Leu	Thr	Tyr	Gly	Asn	Ser	Cys	Ile	Asn	Pro	Leu	Leu	Tyr	Thr	Leu	Leu
305					310					315					320
Thr	Lys	Asn	Tyr	Arg	Glu	Tyr	Leu	Arg	Gly	Arg	Gln	Arg	Ser	Leu	Gly
						325			330						335
Ser	Ser	Cys	His	Ser	Pro	Gly	Ser	Pro	Gly	Ser	Phe	Leu	Pro	Ser	Arg
						340			345						350
Val	His	Leu	Gln	Gln	Asp	Ser	Gly	Arg	Ser	Leu	Ser	Ser	Ser	Ser	Gln
					355			360							365
Gln	Ala	Thr	Glu	Thr	Leu	Met	Leu	Ser	Pro	Val	Pro	Arg	Asn	Gly	Ala
					370			375							380
Leu	Leu														
															385

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Glu	Thr	Pro	Asp	Cys	Phe	Trp	Lys	Tyr	Cys	Val	
1				5					10		

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1539 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGGACAGTGG	GTCCCAATGG	CTCTAGGGTC	CTCCTGTGTA	GCTGGGGAGA	TAACAAAAAA	60
GGGATTCTTT	TGAGGCTTCC	AACAGGATAT	AGGACCTGGT	GAGCCTTTGT	CTCTCTGCAT	120
AGGGACAGTG	ACTGTGTCCA	TCACAGAGGC	TGTTTAGGGC	ATAGAAGTAG	GTTACTGCCT	180
TGAACCTCTG	ACACTAATCT	TTTCCCACAG	GACAAGTTTC	CCACGGGCTC	TCCTCACTGA	240
GCAGTGGTTC	TCCCCCTGGA	ATCCCAAGTGT	GAGGACCGAG	ATGGCTCTGA	GCCTGGAGTC	300
TACAACAAGC	TTTCATATGC	TCACCGTGTG	CGGAAGCACT	GTGACTGAGC	TGCCTGGTGA	360
CTCCAAACGTG	TCCCCTCAACA	GTTCTGGTC	CGGCCCAACA	GATCCCAGCT	CCCTGAAAGA	420
CCTTGTGGCC	ACGGGTGTCA	TCGGGGCAGT	GCTCTCAGCC	ATGGGTGTGG	TGGGCATGGT	480
GGGAAATGTA	TACACTTTGG	TGGTCATGTG	CCGGTTTCTG	CGTGCCTCGG	CCTCCATGTA	540
CGTCTATGTG	GTCAACCTAG	CGCTGGCTGA	TCTGCTGTAC	CTGCTGAGCA	TTCCCTTCAT	600
CATAGCCACC	TACGTCACTA	AGGACTGGCA	CTTGGGAGAT	GTGGGCTGCA	GAGTCCTCTT	660
TAGCCTGGAC	TTCCCTGACAA	TGCACGCCAG	CATCTTCACC	CTGACCATAA	TGAGCAGCGA	720
ACGCTATGCA	GCCGTACTGA	GGCCCTCTGGA	CACAGTCCAG	CGCTCCAAGG	GTTACCGTAA	780
GCTGCTGGTG	CTGGGCACCT	GGTTGCTGGC	ACTGCTGCTG	ACCCCTACCCA	TGATGCTTGC	840
CATCCAGCTG	GTCCCGCAGGG	GCTCTAACAGAG	CCTCTGCCTG	CCAGCCTGGG	GCCCTCGTGC	900
CCACCGTACT	TACCTAACGT	TGCTCTTGG	GACCAGCATT	GTGGGGCCTG	GCTTGGTCAT	960
TGGGCTGCTC	TATGTCCGTC	TGGCCAGGGC	CTACTGGCTA	TCTCAGCAAG	CTTCTTTCAA	1020
GCAGACACGG	CGGCTGCCCA	ACCCCAAGGGT	GCTCTACCTC	ATCCTTGGTA	TCGTCTTCT	1080
CTTCTGGGCC	TGCTTTCTAC	CCTTCTGGCT	GTGGCAGCTG	CTGGCCCAGT	ACCACGAGGC	1140

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CATGCCACTG	ACTCCCGAGA	CTGCACGCAT	TGTCAACTAC	CTGACCACCT	GCCTCACTTA	1200
TGGCAACAGT	TGCATCAATC	CCTTGCTCTA	CACTCTGCTC	ACCAAGAACT	ATCGAGAGTA	1260
CCTACGTGGC	CGCCAGCGGT	CACTGGGTAG	TAGTTGCCAC	AGCCCAGGGA	GTCCTGGCAG	1320
CTTCCTGCC	AGCCGAGTCC	ACCTCCAGCA	GGACTCGGGC	CGCTCGCTGT	CCTCCAGCAG	1380
CCAACAGGCC	ACAGAGACCC	TCATGCTGTC	TCCAGTCCCC	CGTAACGGGG	CCCTTCTCTG	1440
AGAGTGCACT	GTGCAATCCT	GGCATAGGAA	AGGACCCAAA	GGCGTGCGGC	TCCGGAGCGC	1500
ATTTCCCAGA	ATCCCCCTGCT	CAAACCTAAC	TGGCTCGTC			1539

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/US 99/01634

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6	C12N15/12	C07K14/72	G01N33/68	C07K16/28	A61K48/00
	C12N15/11	C12Q1/68			

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N A61K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X, L	EP 0 859 052 A (SMITHKLINE BEECHAM CORPORATION (US); SHABON USMAN; BERGSMA DEREK J.) 19 August 1998 (1998-08-19) L: Priority the whole document ---- -/--	1-23, 25, 27, 29, 31-33



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 1999	20/07/1999
Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Macchia, G

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/US 99/01634

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>MARCHESE A. ET AL.: "Cloning and chromosomal mapping of three novel genes, GPR9, GPR10, and GPR14, encoding receptors related to Interleukin 8, Neuropeptide Y, and Somatostatin receptors"</p> <p>GENOMICS, vol. 29, 1 January 1995 (1995-01-01), pages 335-344, XP002077455 ISSN: 0888-7543 cited in the application page 338, right-hand column - page 339, left-hand column page 343; figure 3C page 344, left-hand column</p> <p>---</p>	1-17, 21-23, 25,27, 29,31-33
X	<p>TAL M. ET AL.: "A novel putative neuropeptide receptor expressed in neural tissue including sensory epithelia"</p> <p>BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 209, no. 2, 17 April 1995 (1995-04-17), pages 752-759, XP002067179 ISSN: 0006-291X cited in the application the whole document</p> <p>---</p>	1-17, 21-23, 25,27, 29,31-33
A	<p>STADEL J.M. ET AL.: "Orphan G protein-coupled receptors: a neglected opportunity for pioneer drug discovery"</p> <p>TRENDS IN PHARMACOLOGICAL SCIENCES, vol. 18, no. 11, 1 November 1997 (1997-11-01), pages 430-437, XP004099345 ISSN: 0165-6147 the whole document</p> <p>---</p>	21-23, 25,27, 29,31-33
A	<p>LEE N.H. AND KERLAVAGE A.R.: "Molecular biology of G-protein-coupled receptors"</p> <p>DRUG NEWS AND PERSPECTIVES, vol. 6, no. 7, 1 September 1993 (1993-09-01), pages 488-497, XP000677175 ISSN: 0214-0934</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/01634

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 18 and 19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Appl. Application No.

PCT/US 99/01634

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0859052 A	19-08-1998	US 5851798 A JP 10295376 A	22-12-1998 10-11-1998